EFFECT OF ANTIPYRETICS ON THE FORMATION OF LEUKOCYTIC PYROGEN BY GRANULOCYTES

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The effect of sodium salicylate, acetylsalicyclic acid, aminopyrine, and phenazone on the ability of granulocytes to produce endogenous pyrogen was studied. Experiments in vitro with verification of the viability of the leukocytes showed that of these antipyretics only sodium salicylate inhibited pyrogen formation.

KEY WORDS: fever; pyrogens; granulocytes; antipyretics.

On interaction with various irritants granulocytes have the property of producing a series of biologically active substances, one of the low-molecular-weight fractions of which is a thermolabile protein pyrogen with high activity. There is evidence to show that this endogenous pyrogen plays the main role in the development and maintenance of fever of varied etiology [3, 5, 13, 15]. In this connection it has been suggested that antipyretics have not only a central action on the temperature-regulating centers [4, 12], but also a "peripheral" action, inhibiting the formation of pyrogen by leukocytes. For instance, sodium salicylate has been shown to inhibit pyrogen formation by leukocytes of blood or peritoneal exudate [7]. Sodium acetylsalicylate and aminopyrine have no such action [1, 9]. In the present investigation the action of various antipyretics on the ability of exudate granulocytes to form leukocytic pyrogen was studied under comparable conditions.

EXPERIMENTAL METHODS

Experiments were carried out on 62 chinchilla rabbits of both sexes weighing 2.2-3.3 kg. Granulocytes were obtained from peritoneal exudate [2, 6]. The animals were given an intraperitoneal injection of 400-500 ml of a 0.2% solution of glycogen in 0.85% NaCl. The exudate containing 85-90% of granulocytes was removed after 18 h. After washing for 2 h at 4°C in pyrogen-free 0.85% NaCl solution, a suspension of granulocytes was prepared with a concentration of 35 million cells/ml. The leukocytes were incubated at 37°C in flasks for 2 h with periodic shaking. The cells were removed by centrifugation at 2000 rpm for 20 min at 4°C, and the supernatant containing leukocytic pyrogen was tested for pyrogenic activity. Antipyretics (sodium salicylate, acetylsalicylic acid, aminopyrine, phenazone) were added to the leukocyte suspension before incubation up to a final concentration of 10 mM, pH 5.7-6.0. The solution of acetylsalicylic acid was first neutralized with $NaHCO_3$. Since the antipyretics were added to the incubation medium and they still remained in the experimental preparations after removal of the leukocytes, in control series of experiments antipyretics were added in the same doses to the final preparation of leukocytic pyrogen before injection into the animal. To assess the possible toxic action of the antipyretics, the viability of the leukocytes was determined before and after incubation by staining with a solution of trypan blue [8]. The pyrogen preparations were injected into the marginal vein of the ear in a volume of 2 ml/kg body weight. The rectal temperature was measured by a resistance electrothermometer at intervals of 30-60 min for 2-3 h. The temperature curves were analyzed by means of a planimeter and febrile indices were calculated for periods of 2 h. During the investigation conditions preventing possible contamination with bacterial pyrogens were observed: sterilization of the glassware at 170°C for 2 h, testing all solutions for absence of pyrogenicity, etc. The experimental results were subjected to statistical analysis by Student's t-test.

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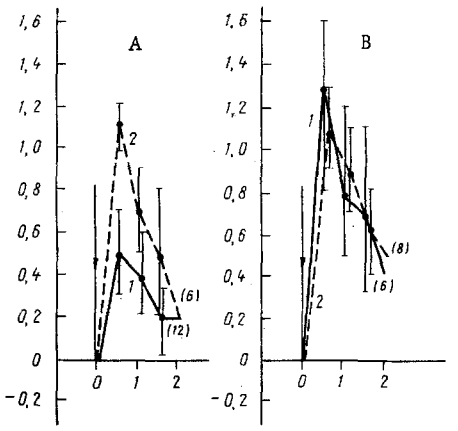

Fig. 1. Effect of sodium salicylate on formation of leukocytic pyrogen by granulocytes and on finished preparation of leukocytic pyrogen. Abscissa, time (in h); ordinate, rise of body temperature (in °C). A: 1) Leukocytic pyrogen obtained after incubation of leukocytes with sodium salicylate; 2) leukocytic pyrogen to which sodium salicylate was added before injection into animal. B: 1) Leukocytic pyrogen incubated with sodium salicylate; 2) leukocytic pyrogen. Arrow indicates time of injection. Vertical lines show confidence limits. Number of animals given in parentheses.

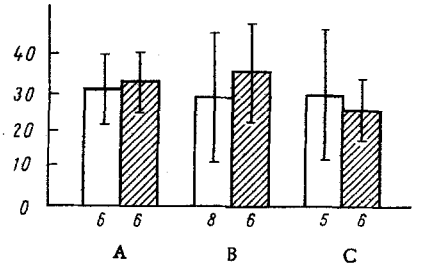

Fig. 2. Ability of granulocytes to form leukocytic pyrogen after treatment with aminopyrine (A), acetylsalicylic acid (B), and phenazone (C). Ordinate, febrile index (in planimetric units). Columns indicate 2-hourly indices of fever induced by leukocytic pyrogen obtained after incubation of leukocytes with antipyretic (unshaded), and by finished leukocytic pyrogen to which antipyretic was added (shaded). Vertical lines indicate confidence limits. Number of animals shown below columns.

EXPERIMENTAL RESULTS

Sodium salicylate considerably inhibited the formation of leukocytic pyrogen by granulocytes of the exudate. As Fig. 1A shows, the temperature reaction developing after injection of leukocytic pyrogen, formed after the addition of sodium salicylate to the incubation medium, was considerably weaker than that caused by the control preparation. The inhibitory action of sodium salicylate was not due to any decrease in the activity of the finished leukocytic pyrogen. The addition of this antipyretic to the pyrogen just before the injection, and also incubation of the finished pyrogen with sodium salicylate for 1.5 h at 37°C, did not alter its pyrogenic activity (Fig. 1B).

The other antipyretics (aminopyrine, acetylsalicylic acid, phenazone), in the doses used, did not affect the ability of the leukocytes to produce pyrogen (Fig. 2). The antipyretics studied have no cytotoxic action on leukocytes. The original suspension contained 95 ± 3% of viable cells, but after incubation for 2 h both under ordinary conditions and on the addition of antipyretics to the medium, the number of viable cells was virtually the same (89 ± 2 and 89 ± 5% respectively, $n_1 = n_2 = 5$).

Of all the antipyretics studied, only sodium salicylate thus inhibited the formation of endogenous pyrogen, in agreement with data in the literature [7]. The mechanism of this action is not sufficiently clear. The present experiments were carried out with leukocytes of an exudate, activated during inflammation, when the basic mechanisms of pyrogen formation had already been triggered and could not be suppressed by blockers of protein synthesis [11]. It can tentatively be suggested that under these conditions sodium salicylate stabilizes the membranes of the leukocytes and reduces liberation of pyrogen. This is in agreement with the data on its stabilizing action on membranes of other blood cells [4].

The central action of antipyretics is well known [4, 12]. The antipyretic effect of acetylsalicylic acid, aminopyrine, and phenazone in fever is probably attributable mainly to this action, for in the present experiments they did not inhibit pyrogen production by exudate leukocytes. It has recently been shown that the central action of antipyretics is connected with depression of synthesis of series E prostaglandins [14], which participate in the mechanism of fever [10, 13], in brain tissue.

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